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(54) Title: THE USE OF MANNAN-RICH MATERIALS IN FEEDSTUFFS

(57) Abstract

Mannan-rich materials such as palm kernel meal or derivatives thereof obtained by breaking down the polysaccharides therein into oligosaccharides are used to promote muscle weight gain, promote the effects of probiotics or to negate the anti-nutritional properties of lectins.

The use of mannan-rich materials in feedstuffs

The present invention relates to the use of mannan-rich materials, and in certain aspects of the invention other oligosaccharide-rich products, in feed products for various purposes set out in more detail below.

The term "feed products" used herein embraces feeds or feed additives for non-human animals as well as foodstuffs or food additives for humans.

The health of animals and of humans can be affected by their intestinal flora, ie. the micro-organisms which are present in their digestive systems. The health of human beings eating animal food may be affected by the intestinal flora of the animal eaten even when the animal itself has suffered no apparent harmful effects. The undesirable effects may result from a micro-organism which is always recognised as a pathogen eg. the cholera vibrio or from a specific strain or excessive amounts of micro-organism which is part of the normal intestinal flora eg. E. coli.

In the case of poultry there is a considerable problem resulting from Salmonella infection, which can affect the health of poultry and of humans consuming poultry and eggs.

In pigs there may be problems with E.coli infection, leading to early mortality among piglets.

It has been proposed to supply bacteria to animals in order to promote the establishment of a non-pathogenic intestinal flora. The bacteria may be supplied to the animal in food or water or may be sprayed onto the animals. These bacterial materials are known as probiotics.

WO 90/14675 refers to the concept of competitive exclusion to prevent Salmonella colonization of poultry. Harmless bacteria are introduced into the digestive tract of birds occupying sites in the digestive system which would otherwise be occupied by Salmonella.

The specific technique which is the subject of WO 90/14675 involves injecting eggs with the bacteria.

suggestion that any marked advantages were obtained. The main benefit was a reduction in cost as the amount of groundnut cake replaced by palm kernel meal.

Work in Thailand has been carried out on feeding pigs on rice bran or palm kernel meal supplemented by amino acids (Warasan Songkhla Nakkhrin 11,1). Again, there is nothing to suggest that the addition of small quantities of palm kernel meal to a pig feed of the type normally used in temperate areas will have any remarkable effect. Fetuga et al, Niger. J. Anim. Prod. 1(2), 132-42 state that pigs fed on PKM grew at a slower rate than pigs on other diets. Work has also been carried out in Malaysia and Nigeria on feeding palm kernel meal to cattle.

Dusterhoft et al, J.Sci. Food Agric disclose that palm kernel meal contains polysaccharides with the major polysaccharides being linear mannans.

A mannan-based oligosaccharide is marketed by the firm of Alltech Inc under the trade name BIO-MOS as a feed additive. It is produced by Saccharomyces cerevisiae when fermenting a complex mixture of sugars. It is described as causing pathogens to clump together so as to reduce their tendency to bind to the wall of the intestine. It also promotes the growth of lactic-acid producing bacteria.

Although chickens, for example, usually carry Salmonella without any harmful effects to the chickens but the presence of Salmonella can adversely affect the economic value of the animal. Various methods have been proposed for preventing or reducing Salmonella infection, the economic effects on animal husbandry and on the incidence of infection of human beings is still unacceptably high.

There is therefore a need for increasing the effectiveness of probiotic agents.

Accordingly, the present invention provides in a first aspect a method of treating an animal to increase its economic value comprising feeding to the animal a derivative of a mannan-rich product and a probiotic.

the kernels. These palm kernels are ground into particulate form and are then processed by either a hydraulic press or solvent extraction to produce palm kernel oil (as distinct from palm oil) and a high protein cake for use as livestock feed. The cake has been prepared by compressing the extracted particles together into the form of a lump. It is, however, dry and gritty and not readily accepted by livestock and the solvent extracted meal with a lower oil content is unplatable and is therefore usually mixed in small amounts with more acceptable and palatable feeds such as molasses.

Guar meal, sometimes known as germ meal, is a well known product and is the residue remaining when guar gum has been extracted from guar seeds. Guar or cluster bean (_Cyamopsis psoraloides_ or _Cyamopsis tetragonolobus_) is a legume grown principally for the gum in the seed coat. It grows in sub-tropical areas such as India, Pakistan, Central America and the southern states of the USA.

Most of the guar crop is used for the production of guar gum, mannogalactan, which is widely used as a gelling agent in human and pet food production as well as the chemical industry. The gum is extracted by a process in which the whole seeds are autoclaved in a continuous flow system. They are then wet milled and the husks with the gum layer floated off. The residue of seed kernels is then centrifuged and dried. The guar gum meal recovered by this process represents about 75% of the original seed,

As explained above mannan-rich vegetable materials in the form in which they are available in commerce are not generally acceptable as feeds for animals, particularly non-ruminant animals. It is therefore desirable to process them in a way which will enable the benefits of using the mannan-rich material at relatively low dose rates.

It is preferred to process the mannan-rich materials so as to convert them from the polysaccharides to manno-oligosaccharides. This conversion may be carried out by treating mannan-containing material with selected enzymes a calf, or may be inherently a mono-gastric animal eg. poultry, pigs, rabbits.

The feed fed to the animal, apart from any probiotic or derivative of a mannan-rich vegetable product which may be fed to it may be a conventional feed for the animal. Poultry feed will normally contain wheat or other grain, a source of protein such as soya, and additives that are well known in the art to provide vitamins, trace elements, and amino acids. Pig feed will normally contain similar components to those mentioned above except that it will be richer in trace elements such as copper and zinc and amino acids such as lysine.

A second aspect of the invention relates to the use of mannan-rich products such as are described above in promoting muscle weight in proportion to other bodyweight, e.g. promoting muscle weight gain.

In rearing animals intended for food it is important to obtain a good gain in weight in response to the feed provided. However weight gain resulting from the accumulation of fat is less valuable than weight gain resulting from the building up of muscle. A reduction in the rate of weight gain, or even a reduction in weight, may be acceptable if the weight of muscle on the animal is increased or maintained.

There is no suggestion in the published literature that palm kernel meal fed in small amounts has any special beneficial effect. In countries which do not cultivate palms and thus do not have large quantities of palm kernel meal to dispose of, there is no incentive to complicate the production of aminal feeds by introducing an ingredient in amounts which are too small to give a significant difference in the cost of the feed.

Accordingly, in a second aspect, the present invention comprises the use of a mannan-rich product or a derivative thereof for promoting increase of muscle weight or in the manufacture of a consumable product for promoting increase of muscle weight. The product according to this aspect of the feed component containing at least one lectin having an anti-nutritional effect in the absence of the second component. The second component containing an oligosaccharide or a derivative thereof which corresponds to a sugar which binds with the lectin to reduce harmful effects resulting from the first component.

There is further included the use of an oligosaccharide-rich product or a derivative thereof in an animal feed to increase the proportion of feed components containing lectins which bind to sugars corresponding to the oligosaccharides and which otherwise have a deleterious effect on the growth of the animal.

This aspect of the invention also includes a method of feeding an animal which comprises feeding it with a oligosaccharide material or a derivative thereof and a feed component containing lectins in an amount sufficient to have an antinutritional effect in the absence of the oligosaccharide-rich material or derivative thereof.

The animal may be a ruminant, but is preferably a non-ruminant. The animal may be a non-ruminant because it is a member of a species which is inherently non-ruminant (mono-gastric), or because it has not yet developed a functioning ruminant digestive system e.g. a calf. Alternatively, the animal may be a bird, for example a galliform such as a chicken, or it may be a mammal, e.g. a monogastric mammal such as a rabbit, or pig.

The feed with which the first and second feed components are used may be any feed which is conventional for the animal being fed.

The anti-nutritional effects of certain feed components are believed to be due to the presence of materials known as lectins. Lectins are commercially available from specialist suppliers as reagents for binding with various sugars. The strength of the binding between a lectin and a sugar will depend on the nature of the lectin and the sugar.

Products Limited. This is the spray dried caecal contents of selected hens. One sachet of the product was mixed with 2.5 litres of water as recommended by the manufacturers, and each bird was sprayed with 0.5 ml. Twenty-four hours later all birds were dosed orally with 104 Salmonella serotype Enteriditis PT4 organisms. The birds were all fed with the same basic feed to which various materials were added, except for two groups used as controls.

The birds were killed humanely seven days later and the caecal contents cultured for Salmonella using brilliant green agar supplemented with nalixidic acid as the plating medium. The growth was scored on a scale of 0-4 after overnight incubation at 37'C.

The scoring system was as follows

0 means < 25 Salmonella per gram
1 means 100 to 1000 Salmonella per gram
2 means 10000 to 100000 Salmonella per gram
3 means 1000000 to 10000000 Salmonella per gram
4 means 100000000 or more Salmonella per gram.

The experiments detailed in Comparative Tests J - L and Example 2 were carried out on newly born black and white male calves, averaging 46.5 kg in weight.

The calves were fed the same feed composition as detailed in Table 2. In addition to this feed, the calves were given a composition as detailed in the Examples below.

The body weight gain and growth rate of the calves were monitored at regular intervals.

The results given are expressed as means. The statistical analyses were performed using the General Linear Model (GLM) procedure of the Statistical Analyses Systems Institutes.

The experiments carried out on Comparative Tests M, N and P and Example 3 were carried out on a group of rats fed on a control diet based on lactalalbumin (LA) as described by Grant et al, The Journal of Food Science and Agriculture 37, 1001-1010 1986. The diet contained 10% of dietary protein and was

30 degrees of polymersiation with a content of about 9% monosaccharide.

The results are shown in Table 1.

Table 1 shows that manno-oligosaccharide did not give a markedly low Salmonella growth score when fed alone, the best (ie. lowest) results were obtained using the combination of probiotic and mann-oligosaccharides.

Comparative Test J

This test was carried out using only the feed as detailed in Table 2. No additive or probiotic was added to the feed.

Comparative Test K

This test was carried out using the feed as detailed in Table 2 supplemented with fructo-oligosaccharide (FOS) (1.5% w/w).

Comparative Test L

This test was carried out using the feed as detailed in Table 2 supplemented with the probiotic Chen Lactobacillus (bovine HSP) (0.01% W/W).

Example 2

This test was carried out using the feed as detailed in Table 2 supplemented with fructo-oligosaccharide (FOS) (0.75% w/w) and the probiotic Chen Lactobacillus (bovine HSP) (0.01% w/w).

The results are shown in Tables 3 and 4.

These results show that the combination of additive and probiotic give better results.

Comparative Test M

This test was carried out on two rats fed on a feed based on Lactalbumin (LA) as detailed above. The E.coli level was 10^3 E.coli/g wet weight.

TABLE 2

FEED COMPOSITION

	Parts by Weight
Skimmed Milk Powder	500
Whey Sweet	283
Nutralys G06B	35
Premix	13
Tallow	36.9
Lard	20
Fish/Coconut	98
Emulbesto SO	9
Berol 696	5
BHT	0.1
TOTAL	1000

TABLE 4

Experiment	Feed	Growth Rate (kg) over period 1-3	3-6	1-6	6-10
J	No additive - no probiotic	5.8	19.4	25.1	32
K	FOS - no probiotic	5.8	18.5	23.5	31.7
L	Probiotic	6.8	20.9	27.7	33.5
2	FOS & probiotic	6.1	21.4	27.6	33.9

The following examples illustrate the second aspect of the invention.

Comparative Test A-2

A group of four rats were fed a control diet based on lactalalbumin (LA as described by Grant et al, 1986, The Journal of Food Science and Agriculture 37, 1001-1010) The diet contained 10% of dietary protein and was fed at the rate of 6g/day for six days. After this time the rats were killed by ether anaesthesia and the weights of the hind lege gastrocnemius muscle determined.

The mean value of the wet weight of the gastrocnemius muscle (mg) per 100g wet body weight was 780.49.

Comparative Test B-2

An experiment was carried out as in Comparative Test A-2 but with 600mg per day of cellulose added to the diet.

The mean value of the wet weight of the gastrocnemius muscle (mg) per 100g wet body weight was 755.70.

Example 1

An experiment was carried out as in Test A-2 but with 600mg per day of material derived from palm kernel meal

TABLE 1-2

Experiment	Feed	Weight- Gain			
		FBW		EBW	
		6 Days	10 Days	6 Days	10 Days
Comp. C	LA	87.3	90.10	81.8	84.6
Comp. D	LA+ cellulose	89.3	91.5	81.7	85.3
Ex. 2	LA+ PKM	90.2	95.6	84.2	88.9

FBW - Final Fresh Body Weight

EBW - Empty Body Weight (FBW-gut contents)

TABLE 2-2

Level of Probiotic (mg/rat/day)	Weight of Gastrocnemius muscle		Growth Rate wt gain/10days
	wet+	dry++	
0	0.95	0.69	12.8
150	0.97	0.71	12.5
300	0.95	0.69	13.0
450	0.97	0.70	13.9
600	1.00	0.75	14.8
	*0.02	*0.03	*14

* Pooled Standard Deviation

+ Castrocnemius wet weight/100g empty body weight

++ Castrocnemius dry weight/100g dry body weight

Comparative Test D-3

The procedure of Comparative Test C-3 was repeated using a feed containing 7% palm kernels and barley plus tapioca, as detailed in Table 4-3. Results taken over a 33 day period are given in Tables 5-3 and 6-3 respectively.

Example 1-3

An experiment was carried out as in Test A-3 but with 600mg of material derived from palm kernel meal added to the diet. The material added was obtained by grinding commercially available palm kernel meal.

The weights mg per 100g wet body weight were mean 549.0 median 607.0-A comparison between the results for Example 1-3 and Comparative Tests A-3 and B-3 shows the effect of the additive of the invention in reducing the harmful effect of kidney bean lectin on the muscle weight of animals.

Example 2-3

The procedure of Comparative Example C-3 was repeated using a feed containing 8% palm kernels instead of rapeseed, as detailed in Table 1-3. Results obtained over 33 days are given in Tables 2-3 and 3-3 respectively.

Example 3-3

The procedure of Comparative Example D-3 was repeated using a feed containing 7% palm kernels and 35% peas as detailed in Table 4. Results obtained over 33 days are given in Tables 5-3 and 6-3 respectively.

TABLE 2-3

AVERAGE BODYWEIGHT

Time	Average Bodyweight (kg±s)	
	Comparative Test C-3	Example 2-3
0	6.8 ± 0.6	6.8 ± 0.6
7	7.7 ± 0.7	7.8 ± 0.7
14	10.5 ± 1.1	11.1 ± 1.0
21	13.7 ± 1.6	15.1 ± 1.7
28	17.0 ± 2.0	19.5 ± 2.1
33	20.8 ± 2.3	23.3 ± 2.3

TABLE 3-3

LIVE WEIGHT GAIN

Time (days)	Weight gain (g/day)	
	Comparative Test C-3	Example 2-3
7	134	147
14	390	472
21	470	572
28	469	621
28-33	761	771
1-33	426	501

TABLE 5-3

AVERAGE BODYWEIGHT

Time (days)	Weight gain (g/day)	
	Comparative Test D-3	Example 3-3
0	6.2 ± 0.7	6.4 ± 0.7
7	7.6 ± 1.1	7.6 ± 1.1
14	10.6 ± 1.8	10.1 ± 1.8
21	14.3 ± 2.5	13.8 ± 2.5
28	18.8 ± 3.0	18.5 ± 3.0
35	24.7 ± 3.7	24.6 ± 3.7

TABLE 6-7

LIVE WEIGHT GAIN

Time (days)	Weight gain (g/day)	
	Comparative Test D-3	Example 3-3
7	205	166
14	426	352
21	629	628
28	554	589
35	852	862
7-35	531	518

8. The use of a mannan-rich product or a derivative thereof or manno-oligosaccharide in promoting muscle weight.

9. A use as claimed in any preceding claim, wherein the mannan-rich product is palm kernel meal or guar meal.

10. A use as claimed in any preceding claim, wherein the mannan-rich product is ground to a particle size of not more than 1000 μm.

11. A use as claimed in any preceding claim, wherein a derivative of a mannan-rich material is used which is produced by the enzymatic breakdown of polysaccharides in said material to produce oligosaccharides.

12. The use of an oligosaccharide in counteracting anti-nutritional effects of lectins.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 94/02802

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF AGRICULTURAL SCIENCE, vol.88, no.3, 1977, UK pages 655 - 661 B.L. FETUGA ET AL. 'The value of palm kernel meal in finishing diets for pigs' see page 655, Summary see page 658, column 2, paragraph 2 - page 660, column 2, paragraph 2	1,8,9
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